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Insect Diversity in Phytoremediation and Bioaccumulation of Se

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ABSTRACT

A variety of plant species are being considered for the phytoremediation of selenium (Se) contaminated soils in agricultural regions of central California. Use of this plant-based technology may also attract a wide range of insects to these Se-accumulating plants. The first field study surveyed the diversity of insects attracted to tall fescue, birdsfoot trefoil, kenaf, and Indian mustard. Over 7500 specimens were collected by a sweep net collection technique for one complete growing season. Most of the 84 families identified were associated with beneficial insects, although pestiferous insects, for example, thrips, aphids, lygus, and leafhoppers, were also found. In the second study the bioaccumulation of Se in the cabbage looper [*Trichoplusia ni* (Hübner)] was investigated on Indian mustard grown in Se-rich water culture solution. Neonate larvae were transferred to plants and fed on Se-treated and no Se treated plants (controls) for 14 days, respectively. Pupae were collected from each treatment and incubated until adult insects emerged. Almost 50% fewer pupae were collected from Se-treated plants compared with "controls", resulting in fewer adult insects. Selenium concentrations were as high as 3173 μg Se kg^{-1} DW in adult insects hatched from Se-treated plants compared with < 5 μg Se kg^{-1} DW in insects from "controls". Based on both studies, we concluded that insect diversity should be determined and insects monitored for bioaccumulation of Se on phytoremediation sites in agricultural regions.

KEY WORDS: bioaccumulation, Indian mustard, *Trichoplusia ni,* selenium.

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I. INTRODUCTION

Several plant species are being considered for the phytoremediation of selenium (Se)-contaminated soils in arid and semi-arid regions of California (Bañuelos and Meek, 1990; Bañuelos *et al.*, 1993; Bañuelos *et al.*, 1997). Plants are selected for their ability to take up large amounts of Se from a Se-laden soil. Because phytoremediation of Se-contaminated soils requires the planting of different plant species in agricultural and thus insect-rich areas of California, identification of insect populations for beneficial or pestiferous species attracted to these plants should be considered. This knowledge is useful for evaluating the potential insect hazard imposed on agricultural crops growing near a remediation site.

Once insects are attracted to a phytoremediation site, little information is available for potential bioaccumulation of Se in insects inhabitating the plants used for phytoremediation. Because phytophagous insects feed on many different organs of plants, the part of the plant consumed by the insect may influence the accumulation of Se by the insect. Possible biotransfer of Se from plant to insect may not only have consequences for the insect (Trumble, Kund, and White, 1998; Vickerman and Trumble, 1999) but also for the invertebrates and vertebrates that feed on them (Wu *et al.*, 1995; Barnum and Gilmer, 1988), if bioaccumulation of Se has taken place (Ohlendorf *et al.*, 1990; Presser and Ohlendorf, 1987). Trumble *et al.* (1998) state the data are lacking that provide information into responses to Se for insects attacking plants that accumulate Se. Such information may become essential for sustainable phytoremediation programs under field conditions.

The objectives of this investigation first were to survey the insect diversity on different plant species (tall fescue, birdsfoot *trevoil,* kenaf, and Indian mustard) commonly used in the field phytoremediation of Se and secondly to investigate the potential bioaccumulation of Se in a major insect pest: cabbage looper incubated on Indian mustard growing in a Se-rich water culture solution.

II. METHODS AND MATERIALS

A. Study 1

A 1-year insect survey was conducted in 1994 on field plots in Los Banos, California. Preplant total soil Se concentrations were approximately 1.5 mg Se kg^{-4} soil at the 0 to 45 cm depth for all plots. Treatments consisted of the following plant species grown only on Se-tainted soil plots: *Brassica juncea* (Indian mustard), *Festuca arundincea* (tall fescue), *Lotus corniculatus* (birdsfoot trefoil), and *Hibiscus cannibinus* (kenaf); soils with lower Se concentrations (controls) were not available at study site. The experiment treatment design was completely randomized with each treatment replicated three times. All plots (each plot was 20 m^2 in size) were sprinkle-irrigated based on estimated potential evapotranspiration reported by the local California Irrigation Management Information System (CIMIS) weather station (Howell *et al.*, 1983). The following plant densities were used for each species: Indian mustard and kenaf (24 plants/m^2), tall fescue, and birdsfoot trefoil (125 to 150 plants/m^2). Forty-five days after plant emergence (June 15th), each plot was sampled weekly at midmorning using a sweep net. Insect sweep samples were taken by the same person over a period of 130 days based on growth stages, that is, flowering, leaf abscission, for the different plant species. There were a total of 15 sampling dates throughout the designated growing season (June 15th to September 15th). Samples were collected by sweeping the plot diagonally through the center of the plot from one corner to the opposite corner. In this study, we assumed that the sweepnet collection technique is influenced by variations of plant morphology (i.e., birdsfoot trefoil-low lying, close to soil surface vs. kenaf-tall, larger leaves, individual plants), behavior of the insect species, weather conditions, and even technique used by individual making collections. Moreover, sweepnets also collect insects that are just visiting or resting on plants. Pedigo and Buntin (1994) describe in detail important considerations for sampling methods for arthropods in agriculture.

The insects collected from each plot were placed into a glass jar, labeled, and stored frozen. At a later date, insects were separated from any plant material and placed into 95% ethanol for later identification. Because of the large number of insect specimens collected, they were identified only to the level of the family of the individual insects represented in the many samples. The predominant species found in each family on each test plant species were identified wherever possible. Plant samples were collected in two 60-day intervals by sampling two 1-m^2 areas within each plot and compositing plant material from each species. Shoot samples were washed with deionized water, oven-dried at 50°C for 7 days, weighed, and ground in a stainless steel Wiley mill equipped with a 0.83-mm screen. Tissue Se was determined by atomic absorption with an automatic vapor accessory after wet acid digestion with $HNO_3/H_2O_2/HCl$ (Bañuelos and Akohoue, 1994). All measurements were made at the most sensitive absorption line (196.0 nm). The NIST Standards Wheat Flour (SRM 1567; Se content of 1.1 ± 0.2 mg kg^{-1}, 94% recovery) was used as external quality control for Se analysis of plant samples.

B. Study 2

Bioaccumulation of Se in the cabbage looper [*Trichoplusia ni* (Hübner)] was investigated with Indian mustard in greenhouse water culture. Indian mustard was selected because it has been identified as the best accumulator of Se among the plants used for phytoremediation of Se in other studies (Bañuelos and Meek, 1990), while the cabbage looper is considered to be a destructive insect to *Brassica* foliage (Ekbom, 1995).

Seeds were sown into shallow flat trays filled with vermiculite potting mixture and then transplanted as 3 to 5-day-old seedlings into 4-L pots filled with a 0.5 modified Hoagland nutrient solution no. 2 (Hoagland and Arnon,1950). There were a total of 20 pots completely randomized with 10 replications per treatment. Treatments consisted of growing Indian mustard in a water-culture solution containing either Se (1 mg Se L^{-1}) or in a no Se (<1 μg Se L^{-1}) solution. Each pot was thinned to two plants after 14 days of growth. Containers were kept in a temperature-controlled greenhouse at 26 ± 2°C during the day and 21 ± 2°C at night, with an average light flux of 450 μmol m^{-2} s^{-1}. Fifteen days after thinning, Se was added as $Na_2SeO_4^{-2}$ to achieve a final solution concentration of 1 mg Se L^{-1} (selenate was selected among the various Se species because it is usually the most plant available form of Se present in the soil and/or effluent). The Se-spiked Hoagland solution was replaced every 7 days, and pH adjusted to 6.7 to 6.9. Deionized water was added to the pots daily to replace water lost by transpiration and to maintain the original volume. Upper sections of each growing pot were snuggly enclosed with clear polyethylene insect cages (approximately 80 cm in height; completely enclosing plants) containing two 10 × 6 cm cheesecloth sections (for air exchange). The lower section of the cage screwed into a female receptor specially attached to top of each growing pot.

After plants were exposed to Se-enriched solution for 10 days, a total of five neonate larvae were transferred to plants growing in each pot with a fine brush (eggs, obtained from laboratory cultures maintained by Thermo Trilogy [Wasco, CA], were previously incubated at 28°C and 60% RH with a photo-period of 14:10 hours [light:dark]) and allowed to feed and mature without disturbance. The developing larvae were examined periodically as they fed on the leaves. Fourteen days later, cocoons containing pupae were carefully collected from the plants for each treatment and placed in large clear plastic containers at 28°C, 60% RH with a photo-period of 14:10 hours. After emergence, adults were frozen within 24 hours and analyzed later for their accumulation of Se. Remaining leaves were collected from each replication, composited from each treatment, washed, oven-dried at 45°C, ground in a stainless steel Wiley mill equipped with a 0.83-mm screen, and stored for Se analysis. Three 0.5-mg leaf samples were taken from each composited treatment and all surviving adult insects from each replicate for each treatment were acid digested with the $HNO_3/H_2O_2/HCl$ procedure described by Bañuelos and Akohoue (1994) and analyzed in triplicate for Se by atomic absorption with an automatic vapor accessory. Final volume of $HNO_3/H_2O_2/HCl$ solution was, however, reduced to 15 ml for insect samples [although ratios remained the same as described by Bañuelos and Akohoue [1994]) to improve the accuracy of detecting low concentrations Se in the insect samples. Both plant and insect were analyzed for Se, already described in Study 1.

III. RESULTS

A. Study 1

We collected a total of 7495 insect specimens from the four different plant species used for phytoremediation of Se over the sampling period (Table l). There were at least 84 families identified from the insects collected. The greatest quantity (specimens; n = 3903) and the greatest diversity (families; n = 76) was found on birdsfoot trefoil followed by tall fescue and kenaf; Indian mustard had the least diversity or fewest families found (n = 27; Table 1). A list of the 84 families identified in the samples is shown in Table 2. Most of the families (65) were represented by only one or a few individuals or species (less than 1% of the total), while most of the insects found were from just 19 different families.

The distribution of the 19 major families, which represented at least 11% or more of the total, is shown in Table 3 for each of the four plants tested. Many of the species associated with the families were beneficial insects and are predators (Anthocoridae, Coccinellidae) or parasites (Braconidae) of other pestiferous insects. Other species

TABLE 1. Comparison of the Diversity of Insects Found Among the Four Plant Species Used for Se Phytoremediation under Field Conditions

Plant used for Phytoremediation	Total number of families found	Total number of insects collected[a]
Birdsfoot trefoil	76	3903
Tall fescue	62	1436
Kenaf	38	591
Indian mustard	27	1565

[a] Total number of insects collected from 15 sweeps for each respective plant species.

found in many of these families include species of serious economic importance to many agricultural products (Aphididae, Cicadellidae, Lygaeidae, and Miridae). A listing of the type of insects and of the most common species found in the samples in each family is given in Table 4.

Of particular importance are those pestiferous species found in very high numbers in the 19 major families (Table 3). Leafhoppers (Cicadellidae) were 15 to 23% of the total in samples taken from birdsfoot trefoil, tall fescue, and kenaf. The leaf beetles (Chrysomelidae) exceeded 12% on Indian mustard and large numbers of the false chinch bug (*Nysius raphanus*, Lygaeidae) were found on Indian mustard (28%) and on kenaf (13%). Species of lygus bugs (Miridae) are serious pests and were found 28% of the total on birdsfoot trefoil, 22% on kenaf, and 37% on Indian mustard. Phytophagous thrips (Thripidae) occurred in high numbers on all of the plants (8 to 28%). Phytophagous leaf beetles (Chrysometidae) were found in abundance only on Indian mustard. Aphids (Aphididae) were also found in moderate numbers on birdsfoot trefoil and tall fescue.

Pest species of note, but that were found in relatively low numbers, were the salt-marsh caterpillar (*Estigmene acrea*, Arctiidae), the plant-hoppers (*Peregrinus* sp., Delphacidae), and others such as the leaf miner flies (*Agromyza* sp., Agromyzidae), which feed inside leaves, stems or roots, and species of root maggots and seed maggots, such as the cabbage-maggot (*Delis radicum*, Anthomyiidae).

Also of primary importance are the many beneficial species that were collected. Of note are predators, including *Aeolothrips* sp. (Aeolothripidae), the assassin bugs (*Zelus* sp., Reduviidae), the minute pirate bugs (*Orius* sp., Anthocoridae), and the ladybird beetles (*Hippodamia* sp., Coccinelidae). Among the parasites found were members of the Braconidae. Collected insects were not analyzed for Se, as they had been preserved in ethanol. Mean plant Se concentrations and standard deviations in leaves collected from all plots two times during the growing season were as follows for the different species in mg Se kg^{-1} dry weight (DW): Indian mustard, 8 ± 2, kenaf, 3 ± 1, birdsfoot trefoil, 3 ± 1, and tall fescue, 2 ± 0.5.

TABLE 2. List of Families Found in Insect Samples Collected from Four Plants Species used for Phytoremediation under Field Conditions[a]

Acrididae	Coreidae	Mycetophilidae
Aeolothripidae[b]	Curculionidae	Mymaridae
Agromyzidae	Delphacidae	Nabidae
Alydidae	Diapriidae	Noctuidae
Anthicidae[b]	Dolichopodidae	Onychiuridae
Anthocoridae[b]	Drosophilidae	Pentatomidae[b]
Anthomyiidae	Dryinidae	Perilampidae
Aphididae[b]	Empididae	Pieridae
Apidae	Encyrtidae	Psyllidae
Arctiidae[b]	Endomychidae	Pteromalidae
Baetidae	Ephydridae	Pyralidae
Berytidae	Eucoilidae	Reduviidae[b]
Bethylidae	Eulophidae	Rhopalidae[b]
Braconidae[b]	Eupelmidae	Sarcophagidae
Calliphoridae	Eurytomidae	Sciaridae
Carabidae	Formicidae[b]	Sepsidae
Cecidomyiidae	Gelechiidae	Sphaeroceridae
Ceraphronidae	Gryllidae	Sphecidae
Ceratopogonidae	Halictidae	Staphylinidae
Chalcididae	Ichneumonidae	Syrphidae
Chironomidae	Lathridiidae	Tachinidae
Chloropidae[b]	Lonchaeidae	Tenebrionidae
Chrysididae	Lygaeidae[b]	Thripidae[b]
Chrysomelidae[b]	Megachilidae	Tingidae
Chrysopidae	Melyridae[b]	Tortricidae
Cicadellidae[b]	Membracidae	Trichogrammatidae
Cixiidae	Miridae[b]	Other or unknown
Coccinellidae[b]	Mordellidae	
Coenagrionidae	Muscidae	

[a] Plant species include: birdsfoot trefoil, tall fescue, kenaf, and Indian mustard.

[b] Families containing 1% or more of the total number of insects found.

B. Study 2

Fewer leaves remained on "control plants" compared with Se-treated plants; the larvae had consumed the missing leaves. Leaf samples from Se-treated plants in all three experiments contained a mean Se concentration and SD of 436 ± 32 mg

TABLE 3. List of the Families of Insects Representing 1% or More of the Total Collected from the Four Plant Species that Have Been Used for Phytoremediation of Se Contaminated Soils

	Plant species used for phytoremediation			
Family of insect	Birdsfoot trefoil (% of total)	Tall fescue (% of total)	Kenaf (% of total)	Indian mustard (% of total)
Aeolothripidae	5	2	0	0
Anthicidae	0	1	0	0
Anthocoridae	6	0	1	0
Aphididae	5	9	0	0
Arctiidae	0	0	5	0
Braconidae	1	0	0	0
Chloropidae	0	2	0	0
Chrysomelidae	3	4	5	13
Cicadellidae	15	23	18	3
Coccinellidae	0	0	4	2
Delphacidae	0	3	0	0
Formicidae	0	0	2	0
Lygaeidae	3	3	13	28
Melyridae	1	2	3	1
Miridae	28	4	22	37
Pentatomidae	0	0	1	1
Reduviidae	0	0	2	0
Rhopalidae	0	2	1	3
Thripidae	17	28	9	8
All other	17	18	14	4

Se kg^{-1} DW. Concentrations of Se in leaves from "control" plants were less than 0.5 mg Se kg^{-1} DW for all three experiments. For all three experiments, almost 50% more adult insects (moths) emerged from pupae collected from "control" plants than from Se-treated plants (Table 5). Dry weight per insect varied among three experiments. Mean dry weights and SD were not significantly greater (ANOVA; $P < 0.05$) for adult insects from "control plants" for all three experiments; 45 mg $\pm$ 10 (controls) vs. 38 $\pm$ 6 (Se-treated) (Table 5).

Selenium concentrations and SD were significantly higher ($P < 0.001$) in adult insects emerging from the Se-treated plants compared with "controls" (Table 5). The mean Se concentration in the Se-exposed adult insect for all three experiments was

TABLE 4. Description of the Type of the Most Common Species Found for Each of the Major Families Collected from the Four Plant Species

Family of insect	Type of insect	Species most commonly represented (genus, common name)
Aeolothripidae	Thrips; predacious, beneficial	*Aeolothrips* sp., the black hunter
Anthicidae	Flower beetles; incidental	*Notoxus* sp.
Anthocoridae	Minute pirate bugs; predacious, beneficial	*Orius* sp., minute pirate bugs
Aphididae	Aphids; phytophagous, harmful	*Therioaphis* sp., alfalfa aphid; *Brevicoryne* sp., cabbage aphid
Arctiidae	Tiger moths; phytophagous, harmful	*Estigmene acrea,* saltmarsh caterpillar
Braconidae	Braconid wasps; parasitoid, beneficial	*Bracon* sp.; *Ephedrus* sp.
Chloropidae	Grass flies; some harmful, some beneficial	*Thaumatomyia* sp.; *Meromyza* sp.
.Chrysomelidae	Leaf beetles; phytophagous, harmful	*Diabrotica undecimpunctata,* Western spotted cucumber beetle; *Phyllotreta pusilla,* Western black flea beetle

Cicadellidae	Leafhoppers; phytophagous, harmful	*Empoasca* sp.
Coccinellidae	Ladybird beetles; predacious, beneficial	*Hippodamia convergens*, convergent lady beetle; *Phyzobius ventralis*, black lady beetle
Delphacidae	Planthoppers; some incidental, some harmful	*Peregrinus* sp .
Formicidae	Ants; some beneficial, some harmful	*Solenopsis xyloni*, Southern fire ant
Lygaeidae	Seed bugs; some predacious, beneficial; some phytophagous, harmful	*Geochoris bullatus* and *G. pallens*, predaceous big-eyed bugs; *Nysius raphanus*, False chinch bug
Melyridae	Soft-winged flower beetles; predacious, beneficial	*Collops vitta*, two-lined collops
Miridae	Plant bugs; many phytophagous, harmful; many predacious, beneficial	*Lygus elisus*, green lygus bug and *L. hesperus*, brown or Western lygus bug (both phytophagous)
Pentatomidae	Stink bugs; phytophagous, harmful; or predacious, beneficial	*Chlorochroa sayi*, Says stink bug; *Acrosternum hilare*, green stink bug

TABLE 4. Description of the Type of the Most Common Species Found for Each of the Major Families Collected from the Four Plant Species (continued)

Family of insect	Type of insect	Species most commonly represented (genus, common name)
Reduviidae	Assassin bugs; predacious, beneficial	*Zelus renardii,* leafhopper assassin bug
Rhopalidae	Plant bugs; phytophagous, harmful	*Liorhyssus hyalinus,* hyaline grass bug
Thripidae	Thrips; phytophagous, harmful	*Frankliniella occidentalis,* Western flower thrips
All other	Families representing in families with <1% of total	Most represented by only one or a few individuals; these were not identified

TABLE 5. Selenium Concentrations in Leaf and Adult Insects and Dry Weights of Emerged Adults (Moths) Collected from Se-Treated Indian Mustard[a]

Treatment (mg Se kg^{-1} DW)	Leaf Se conc.[b] (mg DW^{-1} insect)	Total emerged moths[c] (#)	Moth dry weight (μg Se kg^{-1} DW)	Moth Se conc. (mg DW^{-1} insect)
Replicate I				
Control	<1	36	47(4)	4(.3)
+ Se	465(12)	16	43(2)	2675(76)
Replicate II				
Control	<1	45	54(3)	2(.2)
+ Se	401(14)	15	40(3)	3173(105)
Replicate III				
Control	<1	34	35(3)	2(.2)
+ Se	443(18)	12	32(2)	2730(82)

[a] Values are the means followed by the standard error in parenthesis from a maximum of nine replications per treatment.
[b] Composite leaf sample from Indian mustard was collected and analyzed from each pot.
[c] Total number of hatched moths collected from all replications for each respective treatment.

2859 ± 273 μg Se kg^{-1} DW. In a preliminary study with cabbage looper larvae (n = 30) feeding on Se-treated plants (data not shown), mean Se concentrations in the collected larvae 7 days later were 2511 ± 185 μg Se kg^{-1} DW. Due to the possibility that undigested leaf material may have remained in the gut of the larvae, collecting larvae for additional analysis of Se was not continued.

IV. DISCUSSION

A. Study 1

These 1-year data provide a useful illustration of the diversity and quantity of insects of economic importance attracted to the four plant species that have been used for phytoremediation of Se-contaminated soils. Continued field monitoring would, however, provide additional information on insect diversity and establishment on plants grown for a longer period of time. Based on the limited data on soil Se at preplant and postharvest and plant Se concentrations measured in the four plant species, as well as having no control soil (low Se concentrations), we cannot make any accurate predictions regarding the role Se may play in changing the attract, repel, or affect suitability of the plant species to herbivores. In addition, the pattern of Se bioaccumulation of the 84 families of insects was not examined. Bañuelos *et al.* (1999; unpublished) are presently examining the potential relationship between soil and plant Se concentrations and Se bioaccumulation in insects frequenting a phytoremediation site supporting Indian mustard.

The insects collected represent a wide range of species from over 80 families, including many beneficial species along with several species of pests. The largest number of insects were collected from birdfoot trefoil and Indian mustard. These two plant species attracted diverse species of arthropods at the time of flowering. If insects are attracted to the volatiles exuding from the flowers of Indian mustard like that reported for canola (Palaniswamy, Gillott, and Slater, 1986), they may lay their eggs on the Indian mustard leaves. Eventually the newly hatched (neonate) larvae will begin feeding on the leaves until their feeding resource is depleted (as observed in "control plants" in Study 2). It is, however, not known whether some insects will migrate to an adjacent agricultural site in search for more food or an alternative source of food or if associations exist between Se concentrations and insect diversity. Alternatively, Long *et al.* (1998) reported that flowering nonagricultural crops (e.g., similar to the plants used for phytoremediation) may provide refuge for beneficial predacious and parasitic species.

Pest species, however, found on the plants may be of concern to other crops. One such group that was found in large numbers is the leafhoppers. A species of particular concern is *Circulifer tenellus*, the beet leafhopper. Bañuelos *et al.* (1992) expressed potential concern about using *B. juncea* for phytoremediation of Se, because it may become a host plant for beet leafhopper, which is a vector for beet curly top virus. Although *B. juncea* may be attractive to beet leafhopper, Bañuelos and colleagues found that it was not a host for the virus. Other insects found in the field collection site that are of economic concern included the lygus bugs (*Lygus elisus* and *L. hesperus*), a species that feeds on seed of the plant pods (Ekbom, 1995). If plants used for phytoremediation harbor large populations of lygus bugs, harvesting of the plants may result in their migration to neighboring agricultural fields, for example, cotton, alfalfa, lettuce, or other crops.

Plants introduced to sites for the purpose of phytoremediation may influence the diversity of arthropod species in surrounding agricultural fields. We are not only interested in the kinds of insects that may be attracted to these plants, but also in the likelihood of a transfer of Se from the plant to the phytophagous insects. Because larvae of the cabbage looper are voracious *Brassica* foliage feeders, the results from feeding trials in Study 2 indicates a potential transfer of Se from the plant to the insect (bioaccumulation of Se). In addition high plant Se concentrations affect the feeding patterns of cabbage looper larvae.

B. Study 2

Overall, there was a lower number of pupae collected from Se-treated plants compared with "control plants" at the end of the study. There was an abundance of leaf tissue still intact on the Se-treated plants compared with little-remaining leaf tissue on "control plants". Consequently, the larvae likely had inadequate food on the control-grown plants; mortality rates would likely have been lower on these plants if we had used fewer larvae. Five larvae were selected to ensure the survival of a sufficient number of adult insects to analyze. On the Se-treated plants, the larvae may have avoided the leaves because of the high Se concentrations in the leaf (>400 mg Se kg^{-1} DW). Selenium in the leaf may have functioned inadvertently as a deterrent for the larvae as observed by Vickerman and Trumble (1999) with *Spodoptera exigua*. Unpublished data by J. A. Johnson (1998) indicated that Indian meal moth larvae (*Plodia interpunctella*) avoided a Se-rich diet (>100 mg kg^{-1} DW). In this regard, Trumble *et al.* (1998) reported that quantity and the form of Se accumulating in plants are likely to influence the population dynamics (e.g., development and survival) of insect herbivores under controlled conditions. They hypothesized that both reduction in population number and development associated with Se toxicity would limit population size. Studies with other metals, for example, Cd and Hg, have shown that concentrations of metals in the insect *Neochetina eichlornae* declined as concentrations of Cd and Hg increased in the plant (Jamil and Hussain, 1992). They hypothesized that the insects were feeding less on the metal-enriched plants; a type of 'avoidance phenomena' had occurred. Similarly, Boyd, Shaw, and Martens (1994) and Reeves, Brooks, and MacFarlane (1981) reported that a high nickel concentration in leaves of other *Brassica* spp. deterred insects from feeding on the plants.

In the present study, leaf Se concentrations of >400 mg kg^{-1} DW in the Indian mustard apparently discouraged feeding by the loopers. We were not able to ascertain effects of either lower or greater plant Se concentrations on the cabbage looper due to the similar Se concentrations found in Indian mustard for all three replicated studies. Selective feeding on Se-containing plants by other insects has been suggested by Trumble *et al.* (1998). Due to the obvious need for food, the larvae enclosed on the "Se-treated plants" may have resorted to a form of cannibalism. This unexpected type of feeding would result in fewer numbers of surviving pupae on the "Se-treated plants". The Se concentration measured in the adult insects (moths) from the Se-treated plants unlikely caused mortality within the test period, because insect dry weight was not significantly influenced by the Se concentrations within its body. Thus, apparent avoidance of the plant by the larvae contributed to a higher mortality rate compared with larva feeding on control plants. However, dry weight differences may have been greater between treatments had there been more leaf material for the larvae to consume in the control treatment.

Selenium measured in the adult insects collected from the Se-treated plants was likely not a result of leaf Se residing in the digestive system because both larvae and the pupae void gut contents before molting to the next developmental stage. The use of adult insects for Se analysis may be misleading, because it eliminated any larvae that may have accumulated lethal doses of Se, but were unable to successfully emerge as adults. Future studies should also evaluate larvae for bio-magnifying Se up the food chain, because they are commonly eaten and may be more important in Se transfer. A transfer of Se from sediment and vegetation to aquatic insects has been documented in Se-rich areas of Kesterson Reservoir in Central California (Hothem and Ohlendorf, 1989). Similarly, Ortel (1995) has also examined the metal transfer of Pb and Cd from food to larvae and successive stages of *Galleria mellonella* L. (Lepidoptera). Metal transfer from food to herbivorous hymenopteran or lepidopteran species has been documented under both field and laboratory conditions (Gintenreiter, Ortel, and Nopp, 1993; Lindqvist, 1992; Heliövaara and Vaisänen, 1990; Andrzejewska, Czarowska, and Matel, 1990). Whether the cabbage loopers or other phytophagous insects would remain and feed on the Se-rich plants in an open field is not known. Field studies are presently investigating the potential bioaccumulation of Se in insects collected on Se phytoremediation sites.

V. CONCLUSION

Plants used in long-term phytoremediation of Se-laden soils, especially at the time of flowering, should be monitored for insects in agricultural regions. In our first study, the insects collected from tall fescue, birdsfoot trefoil, kenaf, and Indian mustard clearly represent a wide range of species over 80 families, including many beneficial species along with several species of pests. An insect survey of both pestiferous and beneficial species of insects is especially important for a multitude of years in agricultural regions, where insect control is for the utmost importance for sustainable agriculture; a longer period of time will avoid problems with temporal population fluctuations that can confound data. Identification of insect families attracted to certain plants should be a factor to consider for the selection for plants for phytoremediation in agricultural regions, especially if there are a significant number of pest or beneficial species that may have a negative or positive impact on pests in adjacent crops.

More studies are needed to evaluate plants used for phytoremediation for the impact of Se concentrations on phytophagous insects and the organisms that feed on them. In our second study, the bioaccumulation of Se was observed in the surviving cabbage looper confined to a no-choice environment. However, quantity and form of Se accumulating in plants grown on Se-rich soils are likely to influence the population dynamics (e.g., migration) of insect herbivores. Under field conditions, phytophagous insects may avoid feeding on Se-rich crops and thus reduce the likelihood of a biotransfer from plant Se to insect. Conversely, Indian mustard may serve as a trap crop in high Se soils, attracting pest insects and reducing resident pest insect populations from adjacent agronomic crops. Both studies indicate that insect diversity should be determined on plants used for phytoremediation of Se and insects should be monitored for any bioaccumulation of Se.

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